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EVALUATION OF FREE RADICAL SCAVENGING, ANTIINFLAMMATORY AND WOUND HEALING EFFECTS OF *NEPHELIUM LAPPACEUM* LEAF EXTRACT

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Abstract

Nephelium lappaceum also known as Rambutan is a locally grown popular tropical fruit grown in these parts of Asia. 2,2-diphenyl-1-picrylhydrazyl (DPPH) model of *in vitro* free radical scavenging test was performed with leaf extracts. The extract was also tested on wound healing activity using mice excision model. The anti-inflammatory effect was tested in mice using the formalin induced paw licking test model. In the DPPH test, *Nephelium lappaceum* leaf extract at all concentrations was found to cause significant free radical inhibition ($p<0.05$). All concentrations of *Nephelium lappaceum* leaf extract showed significantly ($p<0.05$) high wound contraction on Day 6. In the animal model of inflammation, all doses of *Nephelium lappaceum* showed reduced number of paw licking. In conclusion, *Nephelium lappaceum* leaf extract demonstrated in vitro free radical scavenging, anti-inflammatory and wound healing effects.

Keywords: *Nephelium lappaceum, Free radical scavenging, anti-inflammatory, wound healing*

Introduction

Nephelium lappaceum L. (Family- Sapindaceae), popularly known as 'Rambutan', is an evergreen tree about 10-12 m tall with grey to brown or greyish brown branches commonly found in Malaysia but nowadays grown worldwide [1]. They have numerous hairy protuberances for which they have been named 'Rambutan' derived from the local Malay word '*rambut*' which means "hair". It belongs to the family of Sapindaceae, and is closely related to the fruits of tropical trees such as longan and lychee. Rambutan trees are medium sized tropical evergreen tree and typically grow to 12 to 20 m in height, with straight narrow trunks and dense spreading crowns. The leaves are alternate pinnately compound [2], with 3 to 11 leaflets, each 10 to 30 cm long, on a reddish rachis (leaf stem). The small yellow petal-less flowers are borne in many-branched hairy panicles (clusters) in the axils (where leaf meets stem) or may appear to be terminal (but are not). The fruit is oval to elliptical, 3 to 6 cm long and 3-4 cm broad with a thin, leathery rind covered with tubercles (warty protuberances) tipped with soft, hair-like spines [2]. The fruit ripens to yellow or red on the exterior, within which is a thin layer (less than 1 cm or 3/8 in thick) of white to rose-colored, translucent juicy flesh covering a somewhat flattened oval seed. Ethnopharmacologically, it is used as a stomachic, astringent, anthelmintic. The local people believe to be a good remedy for treatment of diarrhea and dysentery. The leaves are used as topical application for treating headache [2]. Dried fruit rind is used as an ingredient in manufacture of soap. The roots, leaves and bark are used in the manufacturing of dyes. In Malaysia, the bark is used as an astringent for tongue conditions. Research work has been done on the effects of *Nephelium lappaceum* on various parts of the plant such as rind, fruits, seeds and flower on wound healing, anti-oxidant and anti-inflammatory etc. Thus, the present study was performed to determine the effects of *Nephelium lappaceum* leaf extracts on anti-oxidant, anti-inflammatory and wound healing activity.

Methods

Plant materials

Nephelium lappaceum leaves were collected from an orchard in Melaka which commercially sells rambutan fruit in the local market. The leaves were washed under running water to get rid of dirt. Then, they were dried under shade and room temperature for two weeks. The dried leaves were then blended finely into powder and were kept in a beaker and stored in a refrigerator at 8°C until further use.

Preparation of extracts

50 grams of dried leaf powder was soaked in 500ml of ethanol and 500ml methanol in separate beakers. The solutions were left untouched for 24 hours. After 24 hours, the solution was filtered by Whatmann filter paper. After filtration, the solutions were evaporated using water bath at temperature 50-60°C. The crude extracts obtained were stored in a refrigerator at 8°C until further use.

Experimental animals

Male Dolly mice were obtained from MSU animal house. The weight of the mice was about 20 to 30 grams. The mice were kept in MSU animal house maintained at a temperature of 20°C, with 12 hours of light and dark cycle. Commercial pellets and water were provided to the mice. The mice were divided into two groups for wound excision and anti-inflammatory activity. For wound excision activity, mice were further divided into 5 groups with 4 mice each [3]. First group was treated with commercial Dettol cream® (Reckitt Benckiser) as standard group. Second, third and fourth group were test group and treated with topical application of ethanolic extracts at low dose, medium dose and high dose respectively. Fifth group treated as control group with no treatment.

For anti-inflammatory activity, male dolly mice were further divided into 5 groups with 4 mice each. First group was treated with indomethacin as standard drug group. Second, third and fourth were experimental group and treated with 10mg/kg, 50mg/kg, and 100mg/kg of *Nephelium lappaceum* ethanolic extracts. Fifth group was control group with no treatment.

For anti-oxidant activity, six groups of samples were prepared in six test tubes. First sample was 0.3mM of ascorbic acid served as standard group. Second, third, fourth and fifth test tubes served as experimental group with 100µg/ml, 150µg/ml, 200µg/ml and 300µg/ml of the methanolic extract respectively. Methanol was used as control.

Free radical scavenging model

Six test tubes containing samples were prepared as mentioned earlier. 0.1 mM DPPH was prepared. 3ml of DPPH was pipetted into 6ml of samples in each test tube. The sample were prepared with ethanolic extracts at concentration of 100µg/ml, 150µg/ml, 200µg/ml and 300µg/ml respectively. All test tubes were kept undisturbed for 30 minutes. After 30 minutes, the absorbance of each sample were measured using UV spectrophotometer preset to wavelength 517nm. Distilled water was used as blank. All the methods were done in dark room because DPPH is light sensitive chemical. The inhibition percentage was calculated using the following formula [4]:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$: Absorbance of control
A_{sample}: Absorbance of sample

Anti-inflammatory model

The mice were given respective drugs orally. After one hour of treatment, the paw of mice were injected with 1% of formalin using a 26 G x 3/8inch and licking for first five minutes were recorded and tabulated as early phase. Five minutes after early phase, formalin was injected again and number of licking were recorded for 20-30 minutes and tabulated as late phase [5].

Wound excision model

The hair at dorsal part of mice was removed using a commercially available hair removal cream (VEET®, Reckitt Benckiser, Malaysia). About 225 mm^2 size area was marked with tracing paper and marker pen. The wound was excised carefully using surgical blade (Aesculap AG& Co. KG, Tuttlingen, Germany, Surgical Blade No 20). The excised wound was not too deep nor too superficial. The excision day was taken as Day 1. After the wound excision, the mice were treated topically daily (once a day) at low (25% or 2500mg in 10ml), medium (50% or 5000mg in 10ml) and high (75% or 7500 mg in 10ml) concentration of the ethanolic extracts. The wound area (mm^2) was measured and noted. The wound contraction was calculated by the formula [3]:

$$\text{Wound contraction (\%)} = \frac{I_{wound} - C_{wound}}{I_{wound}} \times 100$$

I_{wound}: initial wound area
C_{wound}: current wound area

Statistical analysis

The data was analyzed using Statistical Package for Social Sciences version 23. Results were analyzed by One Way ANOVA followed by post-hoc test Dunnett's test. Mean ± standard deviation was used.

Results

Free radical scavenging model

In the anti-oxidant model, ascorbic acid showed the highest percentage inhibition of 94.23%. This was followed by 300µg/ml of the extract which showed 91.69%. 200µg/ml concentration showed 83.14%), and 150µg/ml concentration with 82.10%. 100µg/ml of *Nephelium lappaceum* extract concentration showed 81.06% percentage inhibition. We can see that the higher the concentration of *Nephelium lappaceum* leaf extracts, the higher the percentage inhibition of DPPH. The p value for each concentration of extract was <0.001 which was significant.

Anti-inflammatory effect

In the anti-inflammatory (anti-nociceptive) test, number of paw licking in early phase for indomethacin was 13, while for 10mg/kg dose of *Nephelium lappaceum* was 39, for 50mg/kg was 30, followed by 100mg/kg dose was 22. The number of paw licking for control group was 63. For late phase, number of licking decreased from early phase. The number of paw licking for indomethacin was 6, while for the 10mg/kg, 50 mg/kg and 100 mg/kg dose of *Nephelium lappaceum* extract was 18, 13, and 11 respectively. The control group showed 37 paw licks. P value of all the doses of *Nephelium lappaceum* was <0.001 which was significant.

Wound healing model

The wound area size of each group was found to decrease. For Dettol group (standard), the wound contraction was increasing from day 2 about 33.11%, day 3 about 43.78%, day 4 was 46.89%, day 5 with 52.89% and day 6 for 58.67%. For low dose concentration of *Nephelium lappaceum* leaf extracts showed increasing value of wound contraction with day 2 (19.11%), day 3 (28.89%), day 4 (32.44), day 5 (39.78%) and day 6 (47.11%). Medium dose concentration of *Nephelium lappaceum* also show

increasing wound contraction, day 2 (23.55%), day 3 (29.56%), day 4 (36.00%), day 5 (42.67%) and day 6 (47.78%). While for high dose concentration also shows big number of increasing wound contraction from day 2 (24.89%), day 3 (33.33%), day 4 (38.00%), day 5 (42.67%), and day 6 (48.00%). However, no treatment group shows small increasing percentage of wound contraction. All concentration of *Nephelium lappaceum* extracts has p value <0.05 which is significant for wound healing.

Discussion

Oxidation is a chemical reaction that involves transfer of electrons from one compound to other [6]. The process of oxidation in the human body damages cell membranes and other structures including cellular proteins, lipids and DNA. Oxidative stress occurs when the important balance of free radicals is disrupted due to excess of reactive oxygen species (ROS) or the depletion or deficiency of anti-oxidants such as glutathione or both. Anti-oxidant is important to reduce free radical and ROS from our body.

Nephelium lappaceum extracts demonstrated high free radical inhibition percentage in the DPPH test. The higher the concentration of *Nephelium lappaceum* extracts, higher is the percentage inhibition of free radicals. The highest concentration of *Nephelium lappaceum* has significantly higher percentage of inhibition when compared to normal group with 91.69%. (p<0.001). *Nephelium lappaceum* extract can inhibit free radical because it contains phenolic anti-oxidants. Chemical structure of phenolic compounds has an important impact on radical scavenging activity [7]. N*ephelium lappaceum* rind extract revealed high phenolic content and low pro-oxidant capacity with very strong antioxidant activity. Rambutan peels have been used for their antioxidant property [8, 9, 10]. The methanolic extract of *Nephelium lappaceum* peels exhibited strong antioxidant properties. Anthocyanins, known to possess high antioxidant activity, were extracted from rambutan pericarp tissue [9]. Rambutan with flavonoid compounds exhibited protective effects on liver against lipid peroxidation [11]. Inflammation is normal and beneficial process that occurs when the body's white blood cells and chemicals protect from microorganism entry and infection. It is characterized by swelling, redness, warmness and pain. The pain or anti-nociceptive effect was investigated using formalin induced paw licking test. This model simulates clinical symptoms of pain, which involves two distinct phases. The first phase, neurogenic pain, occurs 0-5 min after the injection of formalin. Then, after a quiescent period, a second phase, inflammatory pain, occurs 15–30 min post formalin injection. The injection of formalin into the right hind paw causes an immediate and intense increase in the spontaneous activity of afferent C-fiber and evokes a distinct quantifiable behaviour indicative of pain (paw licking/biting). The first phase results from direct stimulation of nociceptors. The second phase, classified as inflammatory pain (15-30 min), is a tonic response resulting from the inflammatory processes generated by the release of inflammatory mediators, such as histamine, serotonin, prostaglandin E (PGE), and bradykinin, or activation of the neurons in the dorsal horns of the spinal cord [12]. In this experiment, all doses of *Nephelium lappaceum* shows significant (p <0.001) antiinflammatory effect when compared to control group. The number of paw licking is reduced when the dosage increases. The number of paw licking in early phase for indomethacin is 13 which is the lowest among all. However, the number of paw licking in early phase is lot higher than late phase which probably means the *Nephelium lappaceum* extract and indomethacin inhibits late phase greater than early phase. In the first phase, the extract failed to inhibit the direct effect of formalin on nociceptors. However, in the second phase, *Nephelium lappaceum* extract produced dose-dependent and significant (*P*<.001) attenuation of formalin-induced nociception which was in line with the finding that methanol extracts of *Nephelium lappaceum* exhibited anti-nociceptive effect [13].

This indicates NSAID-like action. Thus, the extract has anti-inflammatory effect.

Wound healing is a dynamic and highly regulated process of cellular, humoral and molecular mechanisms which begins directly after wounding and might last for years [14]. It is a complex biological cascade of cellular and biochemical events that comprises three phases namely inflammation, proliferation and maturation.

In this study, all concentrations of *Nephelium lappaceum* leaf extracts show significant p value and high percentage of wound contraction when

compared with control group. The higher concentration of *Nephelium lappaceum,* the higher the percentage of wound contraction. The wound percentage at day 6 of low, medium and high concentration is 47.11%, 47.48% and 48% respectively. The enhancement of wound contraction effect of *Nephelium lappaceum* extract may be due to the presence of chemical constituents that induces ibroplasia. Fibroplasia is a process that enhances wound healing. *Nephelium lappaceum* and its flavonoid compounds have the ability to counteract destructive cell damage and re-establish normal wound healing induced fibroplasia by TGF-β1 [7]. Besides, *Nephelium lappaceum* may promote wound healing due to the presence of geraniin that reduces or inhibit hyperglycemia. Hyperglycemia can reduce and slow down wound healing process. Setyawati et al., 2015, [11] reported that *Nephelium lappaceum* contains geraniin which can reduce blood glucose levels and body weight of mice on alloxan-induced diabetes. In addition, *Nephelium lappaceum* have anti-bacterial compounds that can promote wound healing. Antibacterials decrease, kills or prevents multiplication of microorganisms which can worsen the wound healing process. Thitilertdecha, 2008, [15] reported on the antibacterial effects of *Nephelium lappaceum* extract showing activity against five pathogenic bacteria.

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Table 1: Absorbance and inhibition percentage of *Nephelium lappaceum* extracts on free radical scavenging effect

sample	Absorbance	Inhibition percentage (%)	P-value
Ascobic acid (standard)	0.05 ± 0.000	94.23	0.00
100µg/ml *NL*	0.164 ± 0.00088	81.06	0.00
150µg/ml *NL*	0.155 ± 0.00033	82.10	0.00
200µg/ml *NL*	0.146 ± 0.00058	83.14	0.00
300µg/ml *NL*	0.072 ± 0.000	91.69	0.00
Control	0.866 ± 0.000	0	-

Figure 1: Inhibition percentage (%) of anti-oxidant activity of *Nephelium lappaceum* extracts

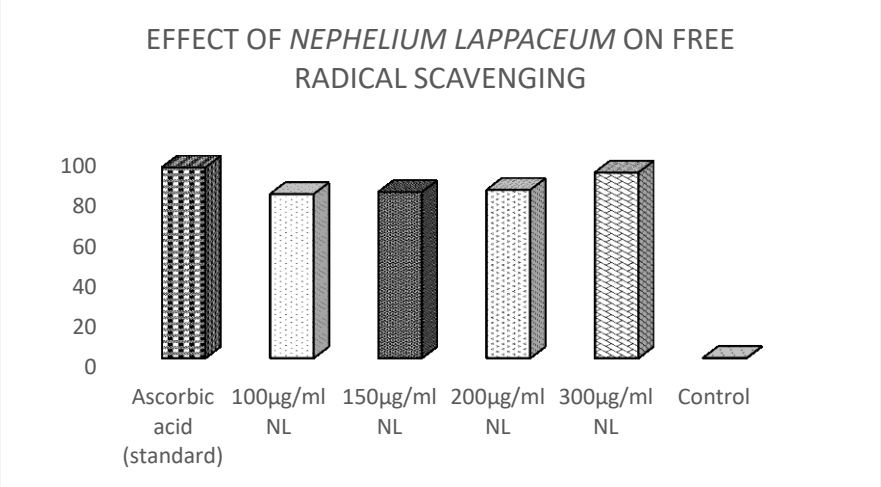

Table 2: Effect of *Nephelium lappaceum* extracts on number of licking on formalin paw licking test

Group	No. of licking		P-value	
	Early phase	Late phase	Early phase	Late phase
Indomethacin (standard)	13 ± 1.11	6 ± 0.58	0.000	0.000
10mg/kg *NL*	39 ± 1.66	18 ± 1.25	0.000	0.000
50mg/kg *NL*	30 ± 1.11	13 ± 0.91	0.000	0.000
100mg/kg *NL*	22 ± 1.08	11 ± 0.86	0.000	0.000
Control	62 ± 3.06	37 ± 1.38	-	-

Figure 2: Effect of *Nephelium lappaceum* extract on formalin paw licking test

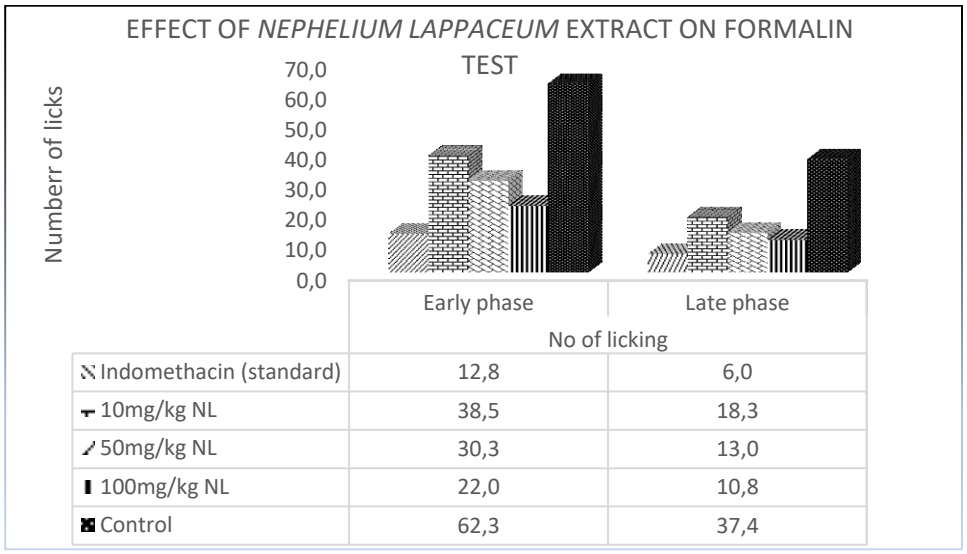

Table 3: Effect of *Nephelium lappaceum* extracts on the wound area (mm^2) ± SEM (wound contraction)

*indicates p value <0.05
**indicates p value <0.001

Group	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Dettol (standard)	225 ± 0 (0)	150.50 ± 10.68** (33.11)	126.5 ± 2.60** (43.78)	119.50 ± 4.91** (46.89)	106.00 ± 3.46** (52.89)	93.00 ± 4.04** (58.67)
Low dose *NL*	225 ± 0 (0)	182.00 ± 3.75* (19.11)	160.00 ± 12.02* (28.89)	152.00 ± 13.86* (32.44)	135.50 ± 14.15* (39.78)	119.00 ± 10.39* (47.11)
Medium dose *NL*	225 ± 0 (0)	172.00 ± 2.02** (23.55)	158.50 ± 2.60* (29.56)	144.00 ± 4.62* (36)	129.00 ± 5.20* (42.67)	117.50 ± 1.44* (47.78)
High dose *NL*	25 ± 0 (0)	169.00 ± 7.51** (24.89)	150.00 ± 5.78* (33.33)	139.50 ± 3.75* (38.00)	129.00 ± 4.04* (42.67)	117.00 ± 4.04* (48.00)
Control	225 ± 0 (0)	217.50 ± 4.33 (3.33)	185.50 ± 2.02 (17.56)	177.00 ± 0.58 (21.33)	162.50 ± 3.75 (27.78)	152.00 ± 1.73 (32.44)

Figure 3: Effect of *Nephelium lappaceum* on wound contraction (%) of from day 1 to day 6